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Polymorphisms in genes related to one-carbon metabolism are not related to pancreatic cancer in PanScan and PanC4

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Abstract

Purpose—The evidence of a relation between folate intake and one-carbon metabolism (OCM) with pancreatic cancer (PanCa) is inconsistent. In this study, the association between genes and single-nucleotide polymorphisms (SNPs) related to OCM and PanCa was assessed.

Methods—Using biochemical knowledge of the OCM pathway, we identified thirty-seven genes and 834 SNPs to examine in association with PanCa. Our study included 1,408 cases and 1,463 controls nested within twelve cohorts (PanScan). The ten SNPs and five genes with lowest *p* values (<0.02) were followed up in 2,323 cases and 2,340 controls from eight case-control studies (PanC4) that participated in PanScan2. The correlation of SNPs with metabolite levels was assessed for 649 controls from the European Prospective Investigation into Cancer and Nutrition.

Results—When both stages were combined, we observed suggestive associations with PanCa for rs10887710 (*MAT1A*) (OR 1.13, 95%CI 1.04-1.23), rs1552462 (*SYT9*) (OR 1.27, 95%CI 1.02-1.59), and rs7074891 (*CUBN)* (OR 1.91, 95%CI 1.12-3.26). After correcting for multiple comparisons, no significant associations were observed in either the first or second stage. The three suggested SNPs showed no correlations with one-carbon biomarkers.

Conclusions—This is the largest genetic study to date to examine the relation between germline variations in OCM-related genes polymorphisms and the risk of PanCa. Suggestive evidence for an association between polymorphisms and PanCa was observed among the cohort-nested studies, but this did not replicate in the case-control studies. Our results do not strongly support the hypothesis that genes related to OCM play a role in pancreatic carcinogenesis.

Keywords

Pancreatic cancer; One-carbon metabolism; Polymorphisms; Biomarkers; Epidemiology

Introduction

Impaired DNA methylation is known to cause cancer through induction of chromosomal instability [1]. Folate and related nutrients (homocysteine, cysteine, methionine, cobalamin, and vitamin B6) are thought to influence carcinogenesis through the one-carbon metabolism (OCM) pathway, which is involved in DNA repair, nucleotide synthesis, and methylation

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[2]. In particular, low folate levels may result in decreased *S*-adenosyl-methionine levels, the primary donor for methylation reactions. It has been hypothesized that hypomethylation may occur as result of altered folate metabolism [3]. One-carbon metabolites (i.e., folate and related nutrients) have been associated with several types of cancer [4–8] and inverse associations with pancreatic cancer (PanCa) have been observed for dietary folate, although study results have been inconsistent [9–16]. In addition, previous studies in association with PanCa for OCM-related SNPs observed a positive association with PanCa for the *MTHFR 677TT* genotype [17] and for two SNPs on the *MTRR* gene (rs162049 and rs10380) [18]. Genome-wide association studies (GWAS) of PanCa have not identified associations with genes involved with OCM [19–24].

In the present study, we used a two-stage approach to evaluate whether 31 genes thought to play a role in the OCM pathway (http://wikipathways.org/index.php/Pathway:WP241) were associated with PanCa. Additionally, genes (*ALPL, CPS1, CUBN, FUT2, PRICKLE2,* and *TCN1*) that included SNPs previously observed to predict one-carbon metabolite levels in GWAS [25–27] were also included. The first stage included nested case control studies from twelve cohorts that participated in the National Cancer Institute (NCI) Pancreatic Cancer Cohort Consortium genome-wide association study (PanScan1) [19]. The second stage, used for replication, included eight case-control studies with GWAS data that participated in the Pancreatic Cancer Case-Control Consortium (PanC4) and were part of PanScan 2 [22].

Methods

The methods for PanScan1 and PanScan2 are described elsewhere [19,22]. The study population included PanCa cases and control participants from the previously conducted GWAS in the PanScan1 and PanC4 [19,22]. The first stage of our analyses included 1,408 incident cases and 1,463 controls from the twelve cohort studies that participated in PanScan1 [19]. The second stage included 2,323 cases and 2,340 controls from eight case-control studies that participated in PanC4 and were part of PanScan1 and PanScan2 [19,22]. All included studies had available dietary and lifestyle data. Cases were defined as participants diagnosed with primary adenocarcinoma of the exocrine pancreas and controls were matched to cases according to birth year, sex, and self-reported race/ethnicity and were free of PanCa at the time of recruitment [19,22]. Genotyping was performed by the NCI Core Genotyping Facility using the Illumina HumanHap550 and HumanHap550-Duo SNP arrays (PanScan1) and Illumina Human 610-Quad arrays (PanScan2) [19,22]. Each individual study obtained informed consent from study participants and approval from local institutional review board. The Special Studies Institutional Review Board of the NCI approved the pooled PanScan study [19,22].

Details of the collection of data across the cohorts are described elsewhere [19]. A total of 37 genes and 834 SNPs were included in the study. From all studies, data on the participant's age and gender were obtained. In addition, five effective principal components or eigenvectors determined from the GWAS data were included as quantitative covariates to correct for population structure.

Data on one-carbon metabolite biomarkers (homocysteine, cysteine, methionine, folate, cobalamin, and vitamin B6) measured in plasma were available for 327 controls of the case-control study nested within the EPIC cohort. Biomarker levels were analyzed by MS-based methods in the laboratory of BEVITAL AS (http://www.bevital.no) in Bergen, Norway [28].

In the first stage, the association for each SNP with PanCa was examined within the cohorts of PanScan using unconditional logistic regression, adjusted for age and sex. To correct for population stratification, analyses were also adjusted for cohort and five principal components of population stratification. Allelic additive models were used where the risk allele was the least frequent allele. *p* values were adjusted for multiple testing using the permutation-based closed step-down minP procedure [29]. Genes were evaluated separately using the adaptive rank truncated product (ARTP) method [30] with 20 truncation points, using 10,000 permutations. This captures potential multiple association signals within a gene, accounting for gene size and LD structure. Gene ARTP *p* values were adjusted for multiple comparisons using a false discovery rate [31].

The ten SNPs and five genes that showed lowest *p* values for the association with PanCa in the cohort data were followed up in eight case-control studies from PanC4, using similar analyses. Because fewer comparisons were performed in this second stage, a Bonferroni correction was applied to both SNP and gene *p* values to adjust for multiple comparisons. Finally, a combined analysis of both stages was performed. Because combined results were mainly driven by cohort results, these were adjusted for multiple comparisons using similar techniques as applied to the first stage.

Adaptive rank truncated product *p* values also were computed for the total OCM pathway in all stages using the top 5 genes as truncation points. An adjusted *p* value below 0.05 was considered statistically significant.

Modification of the top ten SNPs by dietary intake of folate (in tertiles) was assessed in the cohorts by testing the statistical significance of the multiplicative interaction terms using likelihood ratio tests. Differences in least-squares means for plasma metabolite levels (homocysteine, cysteine, methionine, folate, cobalamin, and vitamin B6) were compared per genotype for each SNP by a pairwise *t* test in controls only.

Results

In the first stage of our analyses (cohort studies), rs10887710 (*MAT1A*) showed the lowest *p* value of all tested SNPs (allelic OR 1.24, 95%CI 1.08-1.41, *p* 0.002) (Table 1). However, after correcting for multiple comparisons, none of the SNPs were statistically significant. None of the top ten SNPs for the first stage showed a relation with PanCa in the second stage (case-control studies), with all *p* values greater than 0.2. When all studies were combined (cohort and case-control), statistically significant associations were found for rs10887710 (*MAT1A*), rs1552462 (*SYT9*), and rs7074891 (*CUBN*). However, after correcting for multiple comparisons, the associations were not statistically significant.

Two genes (*MAT1A*, *p* 0.021 and *TYMS*, *p* 0.033) showed statistically significant ARTP P-values in the first stage (Table 2). In the replication stage, none of the genes showed an

association with PanCa. When both stages were combined, *MTRR* (*p* for cohort stage: 0.19) showed a significant *p* value (*p* 0.05). After adjustment for multiple comparisons, no significant associations were found in all stages. Additionally, the pathway analysis showed ARTP-adjusted *p* values above 0.50 in all stages.

There were no significant interactions of the top one-carbon pathway SNPs or genes by dietary folate. Within the subset of EPIC participants with the one-carbon biomarkers, several associations were observed between one-carbon biomarkers and the ten SNPs with the lowest *p* values in the cohort stage. For three SNPs in *TYMS* (rs3819101, rs11873007, and rs3786355) that were in high linkage disequilibrium, the TT genotype correlated with higher methionine levels than the CT (28.46 vs. 25.34 μmol/L; *p* 0.01) or CC genotype (25.45 μmol/L; *p* 0.01). The AA genotype at rs1835898 (*PRICKLE2*) was associated with higher folate (17.26 vs. 14.55 nmol/L for AG; *p* 0.04) and higher pyridoxal phosphate (PLP) concentrations (63.44 vs. 44.45 nmol/L for AG; *p* 0.02 and 41.35 nmol/L for GG, *p* 0.02), when compared to the AG or GG genotypes. The AC genotype at rs222338 (*PRICKLE2*) was associated with higher cobalamin levels than the CC genotype (477.45 vs. 379.26 pmol/L; *p* 0.03) (data not shown).

Discussion

This is the largest genetic study to date examining the relation between germline variations in OCM-related genes and PanCa risk. In the first stage of this study (cohort studies), evidence for an association between genes (*MAT1A* and *TYMS*) and SNPs (most notably rs10887710 [*MAT1A*]) involved in OCM with PanCa was observed. However, after correcting for multiple comparisons, these associations were not statistically significant, nor did the suggestive evidence replicate in the second stage (case-control studies) of this study. No interaction between dietary folate and any of the ten SNPs with lowest *p* values in the first stage was observed.

For several of the top ten SNPs, a correlation with one-carbon biomarkers was observed. This indicates that the SNPs possibly directly influence biomarker levels, which could strengthen evidence for a relation between these SNPs and PanCa. However, none of the SNPs replicated in a second stage and correlations with biomarkers could only be calculated from a subset of all included participants. Additionally, no clear association between one-carbon metabolites and risk of PanCa has been observed within EPIC using the same data on metabolites and PanCa [32].

The reason for observing no replication of the suggestive evidence observed in the first stage of the study may have been due to differences in study designs, as cohort studies may be less prone to survival bias. However, considering the large number of SNPs tested, it is most likely that the significant SNPs observed in the cohort studies, which did not replicate in case-control studies, were false-positives in the first stage of the analysis.

In conclusion, we observed suggestive associations between SNPs and genes involved in one-carbon metabolism and risk of PanCa in the first stage of this study (cohort studies), but none of the results replicated in the second stage (case-control studies). Our results do not

strongly support the hypothesis that genes related to OCM play a role in pancreatic carcinogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Top ten SNPs from cohort with corresponding odds ratios and P-values for cohort, case-control and combined data

Marker[a], Alleles[b], Chrc, Location[c], and Gene[d]	Subset[e]	MAF[f]	Subjects[g]	P value[h]	P value (adjusted for multiple testing)[i]	OR(95% CI)[j]
	Cohort	0.17|0.20	1463|1407	0.002	0.564	1.24 (1.08-1.41)
rs10887710 (T,C), 10q23.1 (82019765), *MAT1A*	Replication, case-control	0.19|0.20	2340|2323	0.341	1.000	1.06 (0.94-1.18)
	Combined	0.18|0.20	3803|3730	0.005	0.990	1.13 (1.04-1.23)
	Cohort	0.02|0.03	1463|1408	0.010	1.000	1.58 (1.12-2.23)
rs1552462 (C,T), 11p15.4 (7213717), *SYT9*	Replication, case-control	0.02|0.03	2338|2320	0.600	1.000	1.08 (0.81-1.44)
	Combined	0.02|0.03	3801|3728	0.033	1.000	1.27 (1.02-1.59)
	Cohort	0.29|0.33	1462|1407	0.011	1.000	1.16 (1.03-1.30)
rs3819101 (C,T), 18p11.32 (667240), *TYMS*	Replication, case-control	0.32|0.33	2336|2323	0.784	1.000	1.01 (0.92-1.11)
	Combined	0.31|0.33	3798|3729	0.098	1.000	1.06 (0.99-1.14)
	Cohort	0.38|0.41	1463|1407	0.013	1.000	1.15 (1.03-1.28)
rs2276433 (C,T), 11p15.1 (20630443), *SLC6A5*	Replication, case-control	0.42|0.39	2340|2323	0.986	1.000	1.00 (0.89-1.12)
	Combined	0.40|0.40	3803|3730	0.800	1.000	0.99 (0.93-1.06)
	Cohort	0.30|0.33	1359|1302	0.013	1.000	1.16 (1.03-1.30)
rs11873007 (C,T), 18p11.32 (670380), *TYMS*	Replication, case-control	0.32|0.34	2326|2309	0.742	1.000	1.02 (0.93-1.12)
	Combined	0.31|0.34	3685|3611	0.100	1.000	1.06 (0.99-1.14)
	Cohort	0.00|0.01	1463|1408	0.014	1.000	2.36 (1.22-4.55)
rs7074891 (T,C), 10p13 (17228480), *CUBN*	Replication, case-control	0.01|0.01	2340|2322	0.356	1.000	1.45 (0.59-3.58)
	Combined	0.01|0.01	3803|3730	0.017	1.000	1.91 (1.12-3.26)
	Cohort	0.45|0.42	1462|1408	0.015	1.000	0.88 (0.79-0.98)
rs1835898 (A,G), 3p14.1 (64096330), *PRICKLE2*	Replication, case-control	0.44|0.44	2339|2323	0.748	1.000	1.01 (0.93-1.11)
	Combined	0.44|0.43	3801|3731	0.172	1.000	0.95 (0.89-1.02)
	Cohort	0.45|0.49	1462|1407	0.016	1.000	1.14 (1.02-1.27)
rs3740873 (G,T), 11p15.1 (20626016), *SLC6A5*	Replication, case-control	0.49|0.47	2340|2323	0.201	1.000	0.95 (0.87-1.03)
	Combined	0.47|0.48	3802|3730	0.646	1.000	1.02 (0.95-1.09)
	Cohort	0.30|0.33	1463|1408	0.016	1.000	1.15 (1.03-1.29)
rs3786355 (C,T), 18p11.32 (671962), *TYMS*	Replication, case-control	0.32|0.33	2338|2320	0.822	1.000	1.01 (0.92-1.11)
	Combined	0.31|0.33	3801|3728	0.125	1.000	1.06 (0.98-1.14)
rs222338 (C,A), 3p14.1 (64202692), *PRICKLE2*	Cohort	0.05|0.04	1463|1408	0.017	1.000	0.73 (0.57-0.95)

Marker[a], Alleles[b], Chrc, Location[c], and Gene[d]	Subset[e]	MAF[f]	Subjects[g]	P value[h]	P value (adjusted for multiple testing)[i]	OR(95% CI)[j]
	Replication, case-control	0.05|0.06	2334|2320	0.751	1.000	1.03 (0.85-1.26)
	Combined	0.05|0.05	3797|3728	0.266	1.000	0.92 (0.79-1.07)

The SNP-level results from the unconditional logistic regression of the genotypes generated in a total of 1,407 pancreatic cancer cases and 1,463 controls.

The analysis adjusted for age in ten-year categories, sex, study, five principal components of population stratification.

[a] NCBI dbSNP identifier.

[b] Major allele, minor allele.

[c] Chromosome and NCBI Human genome Build 36 location.

[d] Gene neighborhood within 20 kb upstream and 10 kb downstream of SNP.

[e] Subset; Cohort: Cohort studies, Case-control: Case-Control studies, Combined: all studies.

[f] Minor allele frequency in control and case participants.

[g] Controls, cases.

[h] 1 d.f. Wald test.

[i] P-values of cohort and combined stage adjusted for multiple testing by the step-down minP procedure. P-values of case-control stage adjusted by Bonferroni.

[j] Estimate assuming additive model

OR, allelic odds ratio; CI, 95% confidence interval.

Table 2

Top five genes from the cohort, with corresponding min P p-values for cohort, case-control, and combined data, and other genes included in the pathway analysis

Gene[a] and Chr[b]	Subset[c]	P value (ARTP)	P value (adjusted)
	Cohort	0.021	0.607
MAT1A, 10q23.1	Replication, case-control	0.255	1.000
	Combined	0.275	0.849
	Cohort	0.033	0.607
TYMS, 18p11.32	Replication, case-control	0.377	1.000
	Combined	0.815	1.000
	Cohort	0.132	0.863
GNMT, 6p21.1	Replication, case-control	0.863	1.000
	Combined	0.832	1.000
	Cohort	0.150	0.863
FUT2, 19q13.33	Replication, case-control	0.546	1.000
	Combined	0.431	1.000
	Cohort	0.188	0.863
MTRR, 5p15.31	Replication, case-control	0.094	0.471
	Combined	0.048	0.595
SLC6A5, 11p15.1	Cohort	0.194	0.863
MTHFS, 15q25.1	Cohort	0.225	0.863
MTHFR, 1p36.3	Cohort	0.230	0.863
FOLH1, 11p11.2	Cohort	0.322	0.863
SLC6A9, 1p33	Cohort	0.333	0.863
GCSH,16q23.2	Cohort	0.342	0.863
TCN2, 22q12.2	Cohort	0.357	0.863
CBS, 21q22.3	Cohort	0.404	0.863
PRICKLE2, 3p14.1	Cohort	0.405	0.863
NBPF3, 1p36.12	Cohort	0.407	0.863
AMD1, 6q21	Cohort	0.430	0.863
CTH, 1p31.1	Cohort	0.432	0.863
MAT2A, 2p11.2	Cohort	0.441	0.863
GIF, 11q13	Cohort	0.500	0.863
CPS1, 2q35	Cohort	0.504	0.863
SHMT1, 17p11.2	Cohort	0.536	0.863
FOLR2, 11q13.3-q13.5	Cohort	0.553	0.863
MTHFD1, 14q24	Cohort	0.564	0.863
SLC1A5, 19q13.3	Cohort	0.590	0.863
GGH, 8q12.3	Cohort	0.623	0.863
ALDH1L1, 3q21.3	Cohort	0.644	0.863
BHMT, 5q13.1-q15	Cohort	0.665	0.863
FOLR3, 11q13	Cohort	0.675	0.863

Gene[a] and Chr[b]	Subset[c]	P value (ARTP)	P value (adjusted)
SHMT2, 12q12-q14	Cohort	0.683	0.863
SYT9, 11p15.4	Cohort	0.700	0.863
CUBN, 10p12.31	Cohort	0.724	0.865
ALPL, 1p36.12	Cohort	0.766	0.886
TCN1, 11q11-q12	Cohort	0.826	0.901
SLC19A1, 21q22.3	Cohort	0.828	0.901
MTR, 1q43	Cohort	0.879	0.929
DHFR, 5q11.2-q13.2	Cohort	0.925	0.951
GSS, 20q11.2	Cohort	0.983	0.983

The gene-level results using the Adaptive Rank Truncated Product approach, of the 37 genes in a total of 1,407 pancreatic cancer cases and 1,463 controls. For each SNP, the logistic regression analysis used adjustment for age in ten-year categories, sex, study, five principal components of population stratification. Adjustment for multiple comparisons was done using an FDR correction for the cohort and combined stage, and using a Bonferroni correction for the case-control stage.

[a]Gene neighborhood within 20 kb upstream and 10 kb downstream of SNP.

[b]Chromosome and NCBI Human genome Build 36 location.

[c]Subset; Cohort: Cohort studies, Replication, case-control: Case-control studies, Combined: all studies.